REMARKS

Interview Summary

Applicants thank the Examiner for the interview between the undersigned and the Examiner on July 15, 2005. During the interview, the Examiner and the undersigned attorney discussed the rejections of the claims in view of the prior art.

Claim Amendments

Currently, claims 17, 18, 38-41 and 43 are pending. Claims 17 and 38 have been amended. Specifically, claim 17 has been amended in step b to recite "comprising the steps of". In addition, claim 17 has also been amended in step b to recite the specific temperatures at which cycle steps b-i) to b-iii) are performed. In addition, in step b-iii) the phrase "but not sufficient to dissociate the primer hybrids" has been deleted in order to make the claim clearer. Support for the temperatures recited in claim 17 can be found on page 6, lines 20-29 and example 5 on page 20, lines 1-3.

Furthermore, claim 38 has been amended. Specifically, in step a) the phrase "to form a reaction mixture in which the amplification primers hybridize with a target nucleic acid sequence and a standard nucleic acid sequence in the test sample" has been deleted in order to make the claim clearer.

No new matter has been added as a result of any of the hereinbefore described amendments.

Brief Summary of the Invention

In order to facilitate the Examiner's understanding of the claimed methods and their differences from the methods of the prior art, a brief summary of the present invention will be provided.

The present invention relates to methods for detecting polymorphisms, specifically, single or large deletions or insertions, in test samples. For large deletions or insertions, the methods of the present invention can be used to detect nucleic acid sequences containing deletions or insertions on the order of 50 nucleotides or more.

One method of the present invention is recited in claim 17. This method allows for the detection of a target nucleic acid sequence in a test sample that is suspected of containing a single or large deletion or insertion. The method comprises the following steps:

a) contacting the test sample with amplification reagents comprising a polymerase, a PCR primer pair, and a probe to form a reaction mixture;

b) performing the following cycle comprising the steps of:

(i) maintaining the reaction mixture for a time and at temperature above 90°C, sufficient to dissociate double stranded nucleic acid sequences,

(ii) maintaining the reaction mixture for a time and at a temperature from 45°C to 65°C to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids,

(iii) maintaining the reaction mixture for a time and at a temperature at least 1°C above the temperature in (ii), sufficient to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid, and

(iv) raising the temperature of the reaction mixture to a temperature sufficient to activate the polymerase;

c) repeatedly performing the cycle of step b) to form an amplification product; and

d) detecting the amplification product as an indication of the presence of the nucleic acid sequence in the test sample.

In this method, steps b-i) to b-iv) of the cycle distinguish this method from other methods known in the prior art. In contrast, the prior art methods employ cycles that contain only three steps. Specifically, these cycles do not employ a separate step b-iii). Applicants have found that performing a cycle comprising steps b-i) to b-iv) improves the resolution of the method, specifically when compared to the three step cycles known in the prior art. This improvement in the resolution of the claimed method is demonstrated in the attached Declaration of Maria C. Gentile Under 37 C.F.R. Section 1.132 (hereinafter "Declaration").

The Declaration describes three (3) experiments that were conducted to detect the presence of the CYP2D6 star five mutation (hereinafter "*5") in human blood samples (See Declaration, Paragraph 4). More specifically, the experiments were conducted to detect the *5 mutation in samples heterozygous for the mutation or homozygous for the non-variant sequence. Each of the experiments was performed exactly in the same manner except for one modification that was made to the PCR cycle (See Declaration, Paragraphs 4-6). In the first experiment described in Paragraph 4 of the Declaration, 45 cycles of the following PCR cycle were performed: 60 seconds at 94°C, 20 seconds at 59°C, 40 seconds at 61°C, and 40 seconds at 72°C. This PCR cycle is illustrative of the cycle claimed in steps b-i) to b-iv) of claim 17. In the second experiment described in Paragraph 5 of the Declaration, 45 cycles of the following PCR cycle were performed: 60 seconds at 94°C, 60 seconds at 59°C and 40 seconds at 72°C. In the third experiment, described in Paragraph 6 of the

Declaration, 45 cycles of the following PCR cycle were performed: 60 seconds at 94°C, 60 seconds at 61°C and 40 seconds at 72°C. The amplification plot for the samples in each experiment (designated FAM-A1-FAM-A9) is shown in Exhibits A (the first experiment), B (the second experiment) and C (the third experiment).

The ability of the claimed method to provide a clear resolution between samples that did not contain the *5 mutation from those that did contain the mutation is shown in Exhibit A. In contrast, as shown in Exhibits B and C, the PCR cycles used in the second and third experiments provided much poorer resolution between those samples. These results demonstrate the benefit of Applicants claimed four step cycle over the three step cycles of the prior art.

A second method of the present invention is recited in claim 38. This method allows for determining whether a deletion or insertion of at least 50 base pairs is present in DNA in a test sample. The method comprises the following steps:

a) contacting the test sample with amplification reagents, wherein the amplification reagents comprise amplification primers;

b) subjecting the reaction mixture to amplification conditions to form a target nucleic acid sequence amplification product, if the target nucleic acid is present in the test sample, and a standard nucleic acid amplification product;

c) detecting a first signal that is proportional to the amount of the target nucleic acid sequence amplification product;

d) detecting a second signal that is proportional to the amount of the standard nucleic acid amplification product; and

e) comparing the first signal to the second signal to determine whether a deletion or insertion of at least 50 base pairs is present in the DNA in the test

sample, wherein the amplification reagents comprise one primer that hybridizes to both the target nucleic acid sequence and the standard nucleic acid sequence

In this method, steps c) to e) distinguish this method from the other methods known in the prior art. Specifically, the methods of the prior art do not involve detecting a first signal that is proportional to the amount of a target nucleic acid sequence amplification product, detecting a second signal that is proportional to the amount of the standard nucleic acid amplification product and then comparing the two signals to determine whether a deletion or insertion of at least 50 base pairs is present in the DNA in the test sample.

In addition to the benefits described above, the claimed methods provide a number of other benefits when compared to the methods of the prior art. First, no single, fully automated platform is currently available in the art for detecting both a single and a large deletion or insertion in a nucleic acid test sample. The claimed methods allow for the creation of such a single automated platform. Second, the methods of the prior art require the running of a gel in order to detect certain types of mutations. The running of gels is both time consuming and expensive. In contrast, the claimed methods do not require the running of a gel to detect the deletions or insertions in a test sample. This saves both time and money.

Rejection of Claims 17 and 18 Under 35 U.S.C. Section 102(e)

Claims 17 and 18 are rejected under 35 U.S.C. Section 102(e) as being anticipated by Wittwer et al., U.S. Patent No. 6,232,079. More specifically, the Examiner states that Wittwer et al. teach a method of claim 17, for monitoring hybridization during PCR for detecting a target nucleic acid sequence in a test sample, comprising:

(a) contacting the test sample with amplification reagents comprising a polymerase, a PCR primer pair, and a probe (the Examiner refers to column 6, lines 1-15, column 44, lines 24-38); and

(b) performing PCR cycles (i) raising temperature to dissociate the double-stranded genomic DNA, (ii) lowering the temperature to allow primers and probe to hybridize to the target nucleic acid, (iii and iv) raising the temperature to dissociate the target-probe hybrids and extending the primers and continuously raising the temperature to permit temperature dependent polymerase extension (the Examiner refers to column 44, lines 50-67, column 45, lines 1-12, column 29, lines 13-36 and column 35, lines 8-31).

In responding to Applicants' previous arguments, the Examiner states that the four temperature cycle of step (b) of claim 17 is broader in scope and does not exclude the temperature cycle disclosed by Wittwer et al. The Examiner says "[S]econd, the disclosure of Wittwer et al. the step of maintaining the reaction mixture for a time and at a temperature sufficient to dissociate the probe hybrid and activating polymerase to a temperature to initiate primer extension occurring simultaneously and repeatedly cycled to form an amplification product which reads on the step (b) of the instant claim 17. Since the instant claims do not recite any specific temperatures at which the step (b) is performed, the instant claims read on temperatures as disclosed by Wittwer et al. Thus the disclosure of Wittwer et al. does not exclude the steps as claimed in the instant claims."

With respect to claim 18, the Examiner states that Wittwer et al. also disclose that the target nucleic acid sequence is a polymorphic nucleic acid sequence.

In view of these arguments, Applicants respectfully traverse this rejection in view of the amendments submitted herewith and the arguments submitted herein.

While not agreeing with the Examiner's arguments made in the Office Action, in an attempt to expedite prosecution, claim 17 has been amended to recite the specific temperatures at which steps b-i) to b-iii) are performed. More specifically, the four steps of b-i) to b-iv) of claim 17 read as follows:

b) performing the following cycle comprising the steps of:

(i) maintaining the reaction mixture for a time and at temperature above 90°C, sufficient to dissociate double stranded nucleic acid sequences,

(ii) maintaining the reaction mixture for a time and at a temperature from 45°C to 65°C to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids,

(iii) maintaining the reaction mixture for a time and at a temperature at least 1°C above the temperature in (ii), sufficient to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid, and

(iv) raising the temperature of the reaction mixture to a temperature sufficient to activate the polymerase;

Claim 18 is dependent upon claim 17.

Wittwer et al. in column 21, lines 22-32, disclose that amplification yields and product specification were optimal when denaturation (93 °C) and annealing

(55 °C) were less than 1 second and that no advantage was found for longer denaturation or annealing times. Wittwer et al. also teach that yield increased with longer elongation times at 77 °C, but that there was little change with elongation times longer than 10-20 seconds. In column 44, lines 50-62, Wittwer et al. describe cycling conditions where denaturation was performed at 94 °C for 0 seconds, annealing was performed at 50 °C for 10 seconds and extension was performed at 72 °C for 0 seconds. This cycle was then repeated 50 times and then cooled to 45 °C.

The cycle steps described by Wittwer et al. are different than the cycle steps claimed in steps b-i) to b-iv) of Applicants method. The difference between the steps of each of the cycles is specifically shown below in Table 1.

Table 1

Cycle steps recited in claim 17	**Cycle steps described by Wittwer et al. in column 44, lines 50-62**
(i) maintaining the reaction mixture for a time and a temperature above 90°C sufficient to dissociate double stranded nucleic acid sequences;	(i) denaturation was performed at 94°C for 0 seconds
(ii) maintaining the reaction mixture for a time and at a temperature of from about 45°C to 65°C to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids;	(ii) annealing was performed at 50°C for 10 seconds
(iii) maintaining the reaction mixture for a time and at a temperature at least 1°C above the temperature in (ii), sufficient to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid; and	
(iv) raising the temperature of the reaction mixture to a temperature sufficient to activate the polymerase.[1]	(iii) extension was performed at 72°C for 0 seconds.

In claim 17, in step b-iii), the reaction mixture is maintained for a time and at a temperature at least 1°C above the temperature in step b-ii) sufficient to dissociate the probe hybrids if the probe is not completely complementary to the nucleic acid (such a probe would be considered to be a "mismatched" probe). Likewise, probe hybrids that are completely complementary to the nucleic acid will not dissociate (such a probe would be considered to be a "matched" probe). Once this step is completed, the temperature of the reaction mixture is raised to a temperature sufficient to activate the polymerase (step b-iv)) and to allow for extension.

[1] The specification on page 6, lines 28-29 states that a temperature sufficient to activate polymerases is typically between 60°C and 90°C, but are "most typically thought to be optimally active at 72°C".

In contrast, Wittwer et al. do not teach a separate step b-iii) in their cycle. Rather, after annealing is performed at 50°C, the temperature of the reaction mixture is immediately raised to 72°C to activate the polymerase and to allow for extension. Thereupon, Wittwer et al. teach the simultaneous dissociation of a mismatched probe and polymerase activation. As shown above in Table 1, in Applicants' method, these cycle steps are separated into two separate steps that are performed under different reaction conditions (namely, at a certain temperature for a certain amount of time). Also, as discussed previously herein, the performance of each of the steps b-i) to b-iv) has been found to improve the resolution of the method particularly when compared to the three step cycle methods of the prior art, such as Wittwer et al. (See the second and third experiments in Paragraphs 5 and 6 of the Declaration).

Therefore, because Wittwer et al. fail to disclose each and every element of the claimed invention, Applicants submit that the rejection of claims 17 and 18 under 35 U.S.C. Section 102(e) in view of Wittwer et al. should be withdrawn.

Rejection of Claims 38-40 and 43 Under 35 U.S.C. Section 102(b)

Claims 38-40 and 43 are rejected under 35 U.S.C. Section 102(b) as being anticipated by Meyer et al., U.S. Patent No. 5,648,482. According to the Examiner, Meyer et al. teach a method of claim 38, for determining deletion or insertion (mutant alleles) of at least 50 base pairs in DNA in a test sample comprising:

(a) contacting the test sample with amplification reagents, wherein the amplification reagents comprise amplification primers, to form a reaction mixture in which the amplification primers hybridize with a target nucleic acid sequence (mutant alleles) and a standard nucleic acid sequence (wildtype) in the test sample (the Examiner refers to column 6, lines 1-4);

(b) subjecting the reaction mixture to amplification conditions to form a target nucleic acid amplification product, if the target nucleic acid is present in the test sample (mutant allele-short fragment) and a standard nucleic acid amplification product (wild-type – long product) (the Examiner refers to column 6, lines 4-14, lines 53-67, column 7, lines 1-7 and column 9, lines 13-40);

(c – d) detecting first and second signal that is proportional to the amount of the target and standard nucleic acid amplification product (the Examiner refers to column 6, lines 6-20, lines 65-67, column 7, lines 1-7, column 9, lines 15-29 and Figure 9);

(e) comparing the first signal to second signal to determine whether a deletion or insertion of at least 50 base pairs is present in DNA in the test sample, wherein the amplification reagents comprise one primer that hybridizes to both the target and the standard nucleic acid sequence (the Examiner refers to column 9, lines 15-25, where she states that SEQ ID NO:1 hybridizes to both mutant and wild-type nucleic acid sequences).

In responding to Applicants previous arguments, the Examiner says in the Office Action that "Meyer et al. does disclose detection of mutations which comprise deletions or insertions of at least 50 bp. The fragments amplified indicate that the standard (wildtype) nucleic acid sequence is 1123 bp and the mutation comprising fragment is 739 bp, which indicates that the fragment of 739 lacks (deletion) a sequence of 1123-739 = 384 bp) which meets the limitation of at least 50 by deletion." Applicants respectfully traverse this rejection.

Applicants submit that the Examiner has misinterpreted Meyer et al. The Examiner states that Meyer et al. disclose the detection of mutations which comprise "deletions or insertions of at least 50 bp". Applicants submit that this is incorrect and that all Meyer et al. disclose are the amplification of distinct fragments and not the detection of a deletion. More specifically, Applicants refer the Examiner to example 2 in column 18 of Meyer et al. Example 2 describes

CYP2D6-specific amplification, particularly the amplification of certain fragments. This example teaches that some mutations of the CYP2D6 (D6) gene in the defective 29-A and 29-B alleles are also present in CYP2D7 (D7) and CYP2DSP (DS) genes of the wild-type allele. In order to exclude "false positive" detection of mutations in pseudogenes, the DNA fragments of the D6 gene containing mutations in the 29-A and 29-B allele were specifically amplified. Six primers were used for the amplification (SEQ ID NOS: 1-6, column 18, lines 44-51). These primers were complementary to certain stretches of the CYP2D6-sequence. The first PCR reaction (CYP2D6-specific amplification) carried out for specific amplification of CYP2D6-specific fragments yielded a 739 bp fragment (fragment B) with primer pair 1 and 2 and a 1123 bp fragment (fragment A) with primer pair 3 and 4. Fragment B contained part of intron 2, exon 3, intron 3, exon 4 and part of intron 4 of CYP2D6 (See column 18, lines 56-57). Fragment A consisted of part of intron 4, exon 5, intron 5, exon 6 and part of intron 6 (See column 18, lines 58-59). Nowhere in this example does Meyer et al. state that a deletion or insertion of 384 bp was detected as alleged by the Examiner. As Applicants discussed in their previous Amendment, Meyer et al.'s method is used simply to detect single or small base mutations (See column 10, lines 28-30, column 15, lines 38-67 and column 17, lines 16-35).

Moreover, Meyer et al. disclose two consecutive amplification reactions (See column 6, lines 56-59). The first amplification reaction amplifies the coding gene without selecting for any mutant variants. The second amplification reaction selectively amplifies the variant alleles and then detects these alleles. Each of these amplification reactions uses different sets of primers in different reaction mixtures depending on the sequence being amplified (See column 9, lines 13-66). In contrast, in Applicants' method, the same primers hybridize to both the target nucleic acid sequence and the standard nucleic acid sequence in a single reaction mixture.

Therefore, because Meyer et al. fail to disclose each and every element of the claimed invention, the rejection of claims 38-40 and 43 under 35 U.S.C. Section 102(b) should be withdrawn.

Rejection of Claim 41 Under 35 U.S.C. Section 103(a)

Claim 41 is rejected under 35 U.S.C. Section 103(a), as being unpatentable over the same Meyer et al. in view of the same Wittwer et al. discussed above. The Examiner states that it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made, to combine the method of amplification of a target nucleic acid as taught by Meyer et al. with the step of primer extension in the presence of a probe or monitoring hybridization during PCR as taught by Wittwer et al. to achieve expected advantage of developing a sensitive and enhanced method for amplification of a specific target. The Examiner also says that the ordinary skilled artisan would have had a reasonable expectation of success. Applicants respectfully traverse this rejection.

The deficiencies of Meyer et al. and Wittwer et al. are stated *supra*. Applicants' arguments are incorporated herein. Applicants submit that Wittwer et al. does not remedy the deficiencies of Meyer et al. and vice versa.

Accordingly, Applicants respectfully request the withdrawal of the rejection of claim 41 under 35 U.S.C. Section 103(a) as being unpatentable over Meyer et al. in view of Wittwer et al.

CONCLUSION

Applicants respectfully submit that the claims comply with the requirements of 35 U.S.C. Sections 102 and 103. Accordingly, a Notice of Allowance is believed in order and is respectfully requested.

Should the Examiner have any questions concerning the above, she is respectfully requested to contact the undersigned at the telephone number listed below. If the Examiner notes any further matters which the Examiner believes may be expedited by a telephone interview, the Examiner is requested to contact the undersigned.

If any additional fees are incurred as a result of the filing of this paper, authorization is given to charge deposit account no. 23-0785.

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